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Spatial distribution of microbial community and N_2O depth profiles in counter- and co- diffusion biofilms functioning simultaneous nitrification and denitrification

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Introduction

Redox stratification in a biofilm where both oxic and anoxic environments are present provides an opportunity for occurrence of simultaneous nitrification and denitrification (SND). Broader application of SND as a small footprint biological nitrogen removal system is contingent on surmounting technical challenges, *e.g.*, control of oxygen supply and mitigation of nitrous oxide (N_2O) emission. However, a conventional SND system faces a difficulty to control oxygen. Furthermore, N_2O emission during SND is favored when oxygen supply for nitrification and organic carbon supply for denitrification are limited (Kampschreur et al. 2009). A conventional biofilm geometry (co-diffusion biofilm), where oxygen and electron donors, *i.e.*, NH_4^+ and organic carbon, co-diffuse from the same direction, affords regions of oxygen-limited and organic carbon-limited conditions, thereby increasing N_2O emission. A biofilm grown on a gas-permeable membrane, *a.k.a.*, counter-diffusion biofilm, promises to overcome the challenges. This configuration allows precise oxygen supply from a gas-permeable membrane on which a biofilm grows (Syron and Casey 2008). NH_4^+ and organic carbon are supplied from the top of the biofilm whereas oxygen from the bottom, creating an environment where organic carbon and NO_2^-/NO_3^-, oxidized by ammonia oxidation at an aerobic region, are confronted. This counter-diffusion biofilm geometry for SND should be, therefore, of advantage to facilitate denitrification including N_2O reduction.

To better understand a mechanism of N_2O mitigation in the counter-diffusion biofilm for SND, this study compared depth profiles of N_2O concentration and spatial distribution of microbial community in the biofilms employing counter- and co- diffusion geometries.

Material and Methods

Two flow-cell reactors with an effective volume of 200 mL were operated for 3 months. One reactor (counter-diffusion biofilm) supplied air via one side of a flat-sheet silicone membrane to the other side in the presence of biofilm whereas the other reactor (co-diffusion biofilm) did from a bundle of hollow-fibers suspended in the bulk liquid. The co-diffusion biofilm had the same geometry with counter-diffusion biofilm including a silicon membrane for biofilm formation; however, an impermeable material was inserted under the membrane, ensuring no oxygen entry from the biofilm bottom. Synthetic medium consisting of CH_3COONa and $(NH_4)_2SO_4$ with concentrations of 200 mgC/L and 200 mgN/L was continuously fed to both reactors at an identical hydraulic retention time (14.5 h). The matured biofilms in both reactors were subjected to O_2 and N_2O microsensor measurements ($n > 5$) with the same methodology previously described (Terada et al. 2010). Subsequently,

they were excised and cryo-sectioned with 100 μm interval by a microtome. Each fraction of biofilm was subject to high-throughput Illumina sequencing of 16S rRNA gene amplicons (Aoyagi et al. 2015). Prediction of denitrifying functional genes from the sequencing libraries was performed by a bioinformatics tool PICRUSt (Langille et al. 2013).

Results and Conclusions

Nitrogen removal efficiencies of the counter- and co- diffusion biofilms were 73.5 ± 4.9% and 63.1 ± 4.0%, respectively, indicating better SND performance of the counter-diffusion geomertry (data not shown). The biofilm depth profiles of dissolved O_2 and N_2O concentrations (Figure 1) demonstrated that the bulk dissolved N_2O concentration in the counter-diffusion biofilm (0.011 mgN/L) was 130 times lower than that in the co-diffusion biofilm (1.44 mgN/L). The far less N_2O production in the counter-diffusion biofilm is congruent with the previous work (Pellicer-Nacher et al. 2010). High-throughput sequencing of 16S rRNA gene amplicons indicate that the classes Alphaproteobacteria, Betaproteobacteria, Actinobacteria and Crostridia were present in both biofilms. The family Nitrosomonadaceae was detected at the aerobic regions. The conversion of the phylogenetic information into functions by PICRUSt suggest that the genes encoding nitrite reductase (*nirK*), nitric oxide reductase (*norB*) and N_2O reductase (*nosZ*) were higher in the middle of the counter-diffusion biofilm whereas higher at both edges of the co-diffusion biofilm (Figure 2). Taken together, biofilm geometry may lead to different spatial distribution and compositions of bacterial guilds responsible for N_2O production and consumption, providing significant difference in N_2O emission.

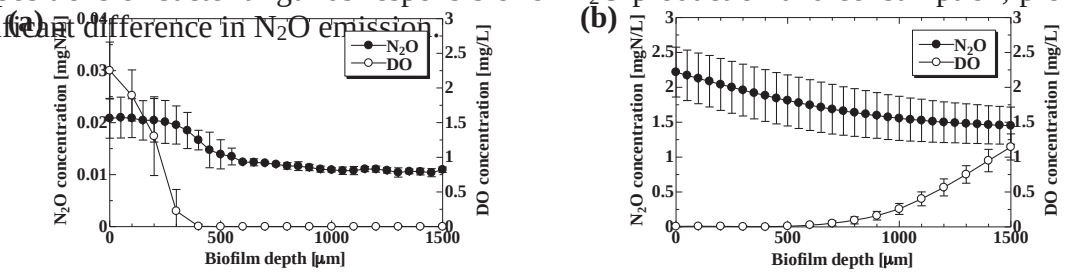

Figure 1 Depth profiles of dissolved O_2 and N_2O concentrations in (a) the counter- and (b) co- diffusion biofilms.

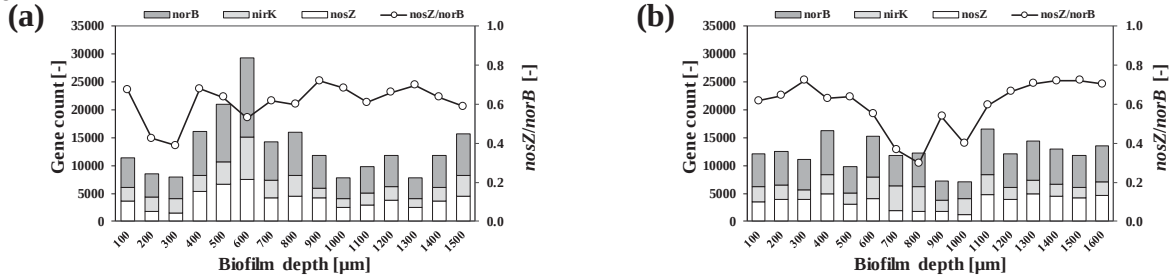

Figure 2 Gene counts of denitrification and relative gene abundance of N_2O consumption over production as a function of biofilm depth with (a) the counter- and (b) co- diffusion geometries.

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